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New neonatal problems of blood coagulation and fibrinolysis

II. Thromboplastic effect of amniotic fluid and its relation to lung maturity

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The presence of surfactant in amniotic fluid has been demonstrated by the shake method [1].
Its quantitative determination is used for an intrauterine diagnosis of lung maturity.
On the other hand, since WEINER [6] reported about the **"Hemostatic activity of amniotic fluid"**, the thromboplastic effect of phospholipids has been high-lighted especially in the obstetric field.

In order to study this effect of the amniotic fluid, we followed gestation by **weekly determination of recalcification time and partial thromboplastin time (PTT) and compared these results with those of the shake method.**

1 Material and methods

In 40 pregnancies we collected amniotic fluid samples via the vagina or by transabdominal puncture, using a silicon treated syringe. Approximately 10 ml were collected and centrifuged for 5 minutes at 1000 rpm. The supernatant was then used for investigations.

With these samples **the following determinations were performed:**

1. Recalcification time.
2. Partial thromboplastin time.
3. Platelet aggregation.
4. Shake test.

1.1 Recalcification time

To 1/2 part of human control plasma (ORTHO), 1/2 part human amniotic fluid was added and after incubation for 2 minutes in a water bath maintained at 37°C, 1 part 1/40 M $CaCl_2$ was added. By using a coagulometer (SCHNITGER und GROSS, West-Germany.) the clotting time was measured.

As control to 1 part of human control plasma, 1 part of 1/40 M $CaCl_2$ was added.

1.2 Partial thromboplastin time (P.T.T.)

To 0.1 ml plasma, 0.1 ml active cephaloplastin (DADE) and after 2 min 0.1 ml 1/40 M $CaCl_2$ were added and the clotting time was measured using a coagulometer at +37°C.

1.3 Platelet aggregation

Platelet aggregation after ADP addition was observed with an automatic recorder (RIKEN, Tokyo, Japan.) connected with an aggregationmeter from EVANS (Great Britain.).
For this investigation to 0.5 ml of P.R.P. (platelet rich plasma) 0.1 ml of 1. 10^{-6} M ADP was added.

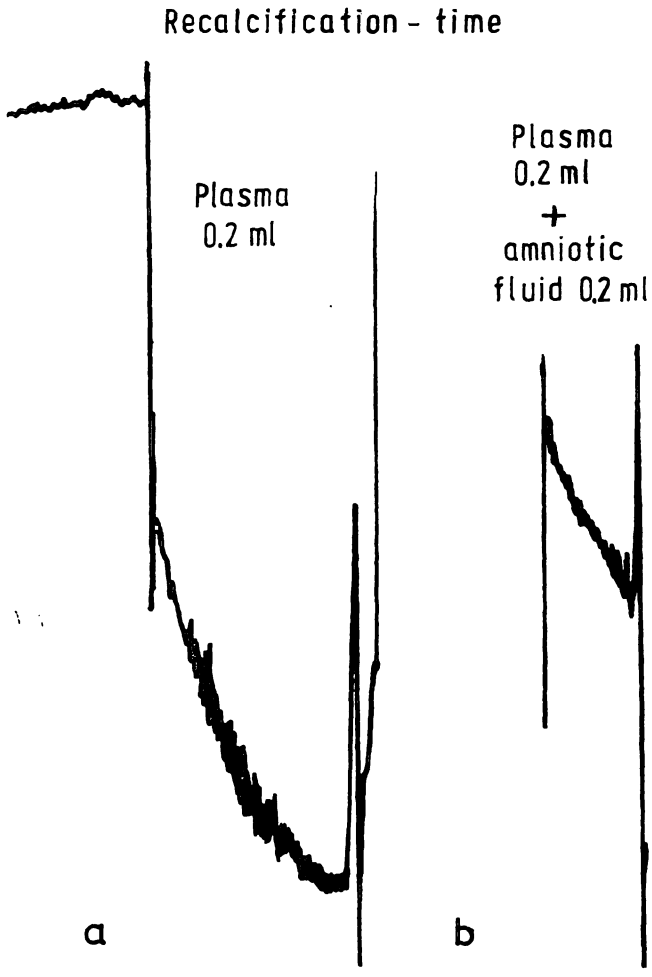

Fig. 1. Recalcification-time of
a) normal plasma
b) normal plasma + amniotic fluid after the 30th gestational week.

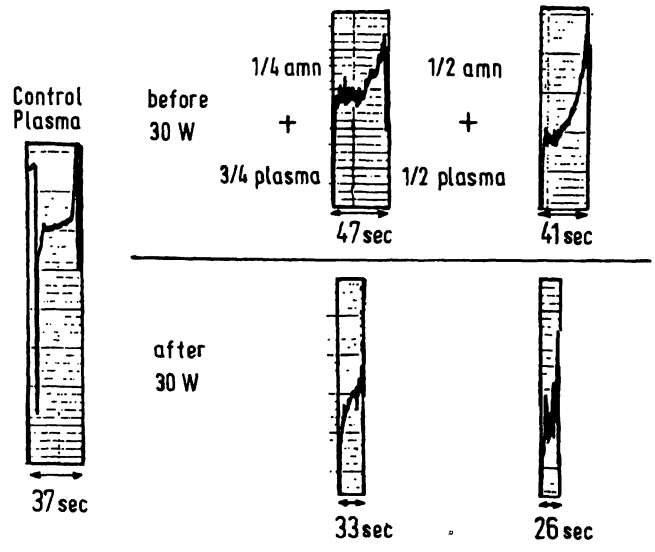

Fig. 2. Partial thromboplastin time of mixtures of standard plasma and amniotic fluid before and after the 30th gestational week.

1.4 Shake test

Lung function was tested in the usual manner by the shaking method according to CLEMENTS et al [1].

2 Results

2.1 Recalcification time: In all of the 15 investigated pregnancies after the 30th week, a shortening of the recelcification time of amniotic fluid with standard plasma was observed, whereas prior to the 30th week no shortening was seen (Fig. 3).

2.2 Partial thromboplastin time: Amniotic fluid exerted a shortening of the partial thromboplastin time of mixture of standard plasma with amniotic fluid after the 30th gestational week.

In early pregnancy amniotic fluid had only little effect (Fig. 2). Before the 30th gestational week a mixture of 1/4 amniotic fluid + 3/4 standard plasma had a coagulation time of 47 sec whereas amniotic of 1/2 amniotic fluid + 1/2 standard plasma clotted within 41 seconds. After the 30th week those mixtures had clotting times of 33 sec and 26 sec. Normal control plasma clotted within 37 sec.

2.3 Platelet aggregation of amniotic fluid after the 30th week caused disagregation of platelets agglutinated by ADP in only 3 cases.

The remaining 15 samples did not cause disaggregation (Fig. 4.).

2.4 The results of shake test is shown Fig. 6.

3 Discussion

The surfactant factor has received considerable attention as a substance which is associated with the degree of maturation of the fetal lung, and due to the possibility that the surfactant factor itself may activate the blood coagulation system.

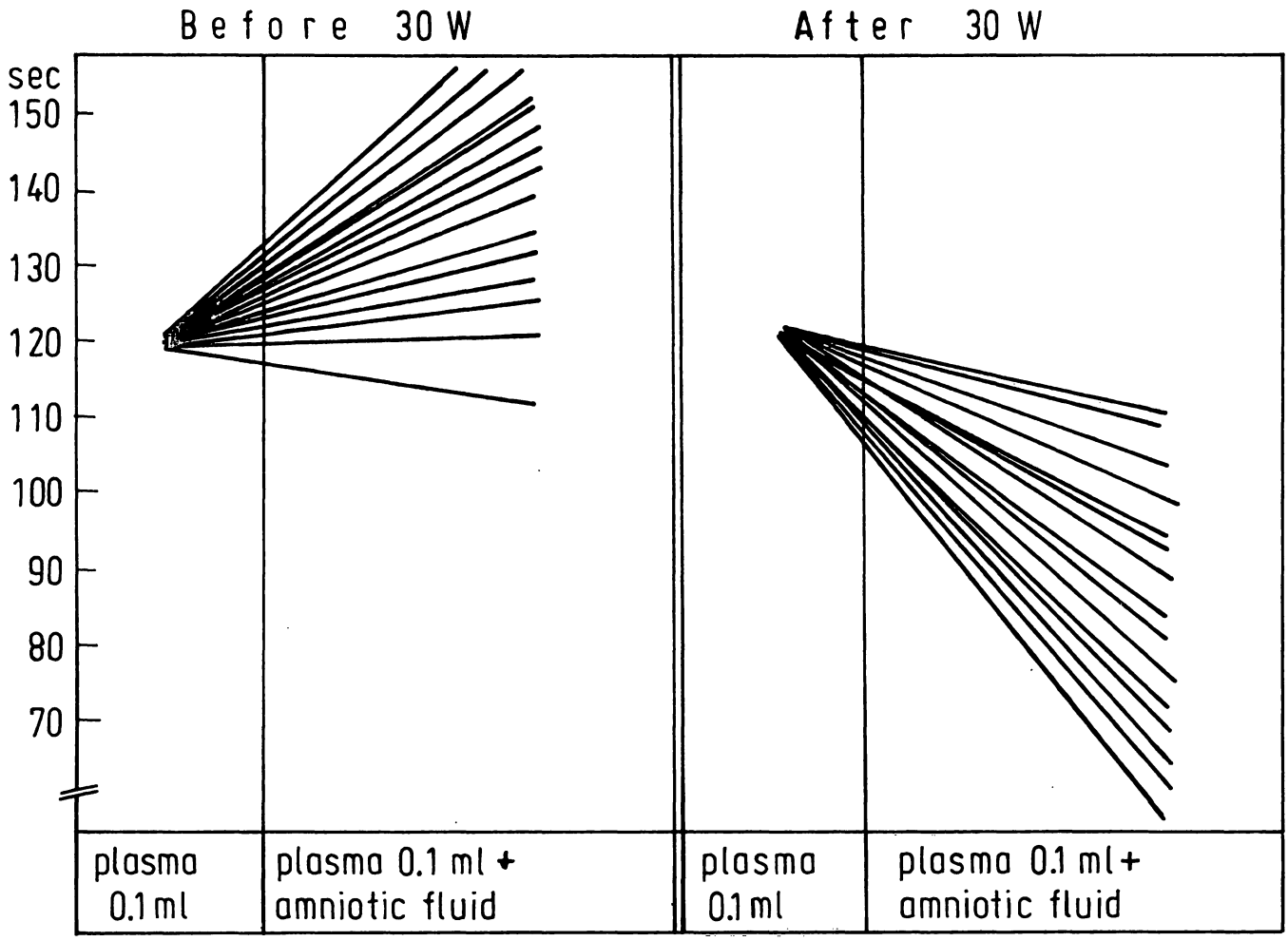

Fig. 3. Recalcification-time of standard plasma after the addition of amniotic fluid during different weeks of pregnancy.

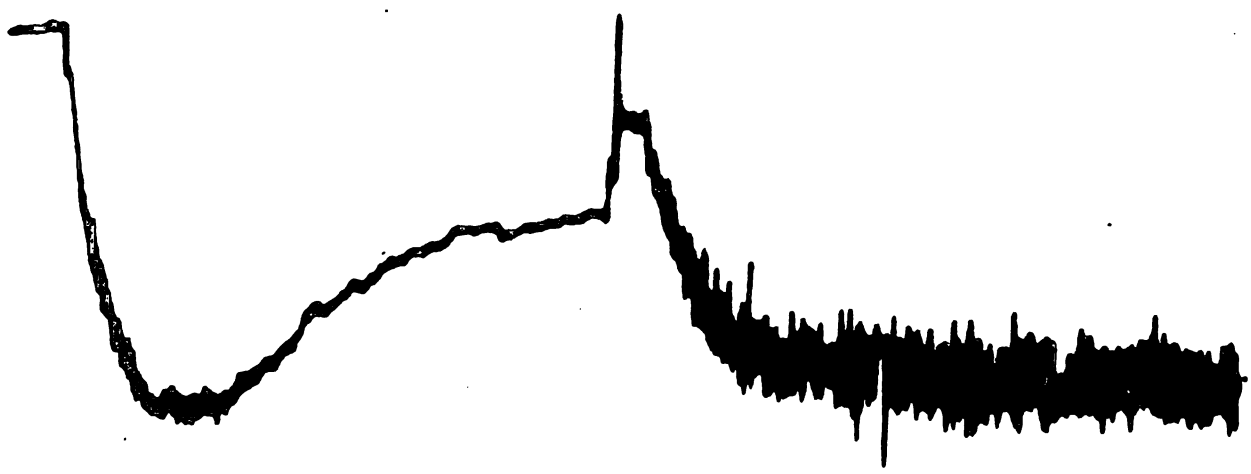

Fig. 4. Platelet aggregation with ADP.
left: normal plasma. disaggregation can be seen.
right: normal plasma + amniotic fluid. (38 week) disaggregation does not occur.

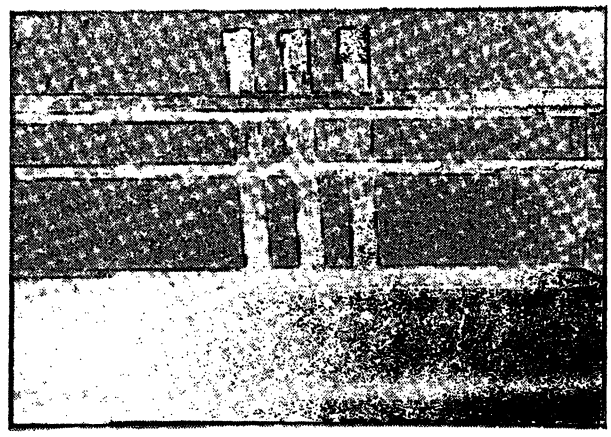

Fig. 5. Shake test (CLEMENTS et al. [1]).

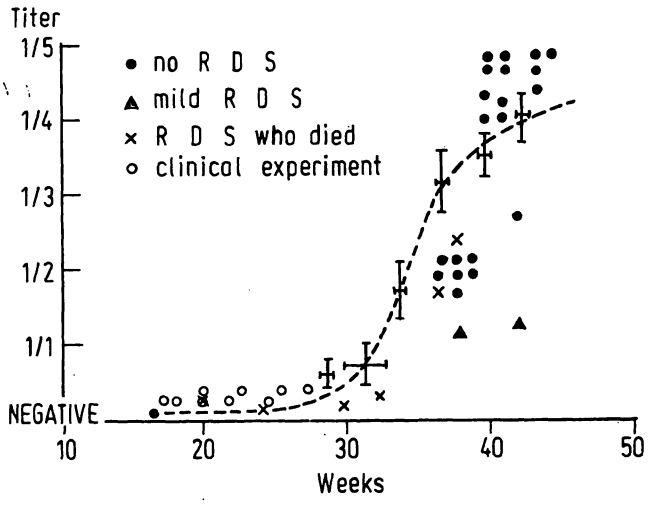

Fig. 6. The results of the CLEMENTS titer.

Regarding this, TAYLOR et al. [3, 4] reported that the surface active lipoprotein which he extracted from lung tissue homogenate plays a role in the mechanism.

Further, COURTNEY [2] compared the amniotic fluid of normal pregnancies with those with fetal death in the uterus (Tab. I.).

As may be clearly seen in Tab. I, in cases of fetal death in the uterus mixtures of amniotic fluid with normal plasma or with Factor VII deficient plasma show a remarkable lengthening of recalcification time.

The explanation could be given according to COURTNEY (Fig. 7.). The changes in the coagulation and fibrinolytic system exerted by the amniotic fluid are associated with the thromboplastic effect which is originated partially by phospholipids.

In this study, the thromboplastic effect of amniotic fluid after the 30th week of pregnancy is shown by a shortening of recalcification time and partial thromboplastin time.

For partial thromboplastin time substances that play the main role in accelerating the clotting process are phospholipids.

By the shaking method according to CLEMENTS et al. and WAKE et al. (2), it was shown that surfactant

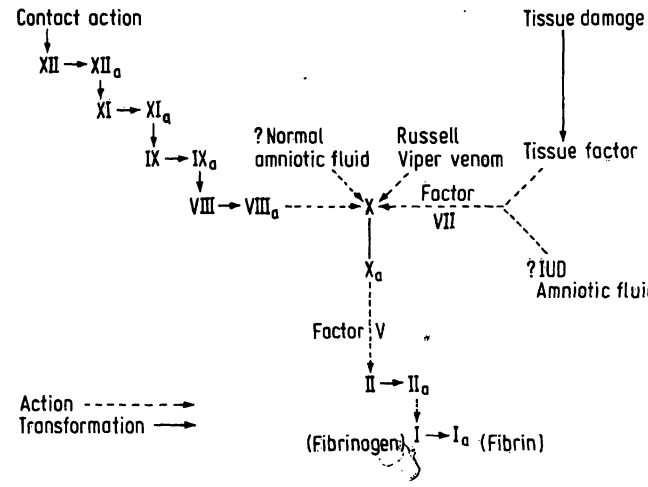

Fig. 7. The possible action of normal and IUD (Intrauterine-death) amniotic fluid on blood coagulation. (CORTNEY [2])

Tab. I. Recalcification-time (COURTNEY [2])

Amniotic fluid	Normal plasma	plasma deficient in			
		Factor VII	Factor VIII	Factor IX	Factor X
IUD 1	65	92	66	86	1000
IUD 2	43	71	48	72	1000
Normal					
Meanso of seven	44	43	42	56	438
range	33–55	38–48	38–50	43–66	540–370

appears in amniotic fluid around the 30th week of pregnancy.

As can be seen in Fig. 6 the so-called CLEMENTS titer [1] gives the same results as our recalcification time and partial thromboplastin time.

Thus the so-called surfactant seems to have enhancing effect on the blood coagulation. Whether this substance is actually α-palmitoyl lecithin or not, must be determined in the future. The detection of the thromboplastic effect of amniotic fluid by coagulation assays can replace the shaking method because it gives the same results.

Further, it is an advantage of this method that the recording can be done with a coagulometer or an aggregation-meter in a semi-quantitative analysis (Fig. 2).

According to our results around the 30th week of gestation should be the borderline for the rise of phospholipids in the amniotic fluid.

They may be detected by the effect they exert on the partial thromboplastin time. Thus, the assay outlined in this paper provides an **effective diagnostic tool for the early detection of surfactants essential for the lung function of newborn infants.**

Summary

Pulmonary hyaline membrane disease in newborn infants is considered an abnormality in the alveolar lining layer.

Quantitative analysis of this surfactant is necessary for the intrauterine diagnosis of lung maturity of the fetus.

The presence of surfactant in amniotic fluid has been demonstrated by the shaking method [1]. But it is also well knowen that amniotic fluid has a thromboplastic effect [3, 6].

In order to compare the correlation between the shaking method and the thromboplastic effect of the amniotic fluid, recalcification time and partial thromboplastin time were measured with and without amniotic fluid using an aggregation-meter.

In each of 15 cases, a shortening of these times was recorded after the addition of amniotic fluid after the 30th week of pregnancy.

In all cases the addition of amniotic fluid resulting in shortening these times.

Surfactant seems to have enhancing effect on the coagulation. These results demonstrate the presence of surfactant in amniotic fluid in agreement with the results of the shaking method. Although these methods are of limited utility as quantitative assays for surfactant, they **are of sufficient accuracy and of great value for clinical diagnosis.**

Keywords: Partial-thromboplastin-time, shaking method, surfactant.

Zusammenfassung

Probleme der Blutgerinnung und Fibrinolyse beim Neugeborenen.
II. Thromboplastischer Effekt des Fruchtwassers und seine Beziehungen zur Lungenreife.

Beim hyalinen Membransyndrom des Neugeborenen kommt es zu pathologischen Entwicklungen der Alveolen. Da die Stabilität der Alveolen weitgehend durch den Gehalt an Phospholipiden, speziell der Lezithine bestimmt wird (Surfaktant) und das Fruchtwasser mit der Lunge intraamnial kommuniziert, kann durch eine Surfaktant-Bestimmung im Fruchtwasser auf die Lungenreife geschlossen werden.

Hierzu dient der Schaumtest nach CLEMENTS [1]. Anderseits ist bekannt, daß Fruchtwasser einen Thromboplastin-Effekt, verursacht durch die Phospholipide, hat. Um festzustellen, ob eine Korrelation zwischen dem Schaumtest und dem Thromboplastin-Effekt des Fruchtwasser besteht, wurden die folgenden beschriebenen Experimente durchgeführt.

a) **Recalzifizierungszeit mit und ohne Zusatz von Fruchtwasser.**
b) **Partielle Thromboplastinzeit mit und ohne Fruchtwasser unter Verwendung eines Agglutinationsmeßgerätes.**

Bei beiden Experimenten wurde in allen 15 untersuchten Fällen eine Zeitverkürzung festgestellt, wenn das Fruchtwasser nach der 30. Woche entnommen worden war.

Fruchtwasser vor der 30 Schwangerschaftswoche ergab keine Zeitverkürzung. Es ergibt sich hier also die gleiche Tendens wie beim Schaumtest.

Die Ergebnisse zeigen, daß die angewandten Methoden, obschon nur halbquantitativ, für die klinische Diagnostik durchaus verwendbar sind.

Schlüsselwörter: Partielle Thromboplastonzeit, Schütteltest, Surfaktant.

Resumé

Nouveaux problèmes de coagulation sanguine et de fibrinolyse en néonatalogie
II. Effet thromboplastique du liquide amniotique et ses rapports avec la maturité pulmonaire

Le syndrome de membrane hyaline pulmonaire chez les nouveauxnés conduit à un développement pathologique des alvéoles.

Or, la stabilité des alvéoles étant largement assurée par la teneur en phospholipides, spécialement en lécithine, (surfactant), et le liquide amniotique communiquant avec le poumon de façon intraamniale, on en déduit qu'il est possible de déterminer la maturité pulmonaire par une analyse quantitative du surfactant dans le liquide amniotique («shaking method» de CLEMENTS et al. [1]).

On sait, par ailleurs, que le liquide amniotique a un effet thromboplastic causé par les phospholipides [3, 6]. Afin donc d'établir s'il existe une corrélation entre la «shaking method» et l'effet de thromboplastine du liquide amniotique, on a mesuré à l'aide d'un enregistreur d'agglutination le temps de récalcification et le temps partiel de thromboplastine avec et sans liquide amniotique.

Dans les deux séries d'examens on a observé sans exception pour les 15 cas un raccourcissement des temps après addition de liquide amniotique prélevé après la 30ème semaine de grossesse, ce qui confirme les résultats enregistrés par la «shaking method» (présence dans le liquide amniotique de surfactant qui accroit l'effet de coagulation).

Bien que ces méthodes ne soient que d'une utilité limitée pour déterminer la quantité de surfactant, elles sont suffisamment précises pour servir au diagnostic clinique.

Mots-clés: «Shaking method», surfactant, temps partiel de thromboplastine.

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